Experimental Crystal Violet and Methyl Violet Poisoning in Dogs and Cattle

by Randall C. Cutlip* and William S. Monlux*

ABSTRACT

Crystal violet has been observed to cause fatal pulmonary alterations in dogs. To further evaluate this toxicity and the toxicity of methyl violet, 12 dogs and 2 calves were given 1 per cent aqueous solutions of the dyes intravenously. Both dyes caused the formation of numerous dye protein emboli which lodged in the lungs producing thrombosis and infarction. A 1 per cent solution of the dyes caused precipitate formation when added to bovine serum or heparinized plasma IN VITRO. Serum proteins in general were decreased as determined by paper electrophoresis of serum-crystal violet supernatants. These dyes could be used effectively in studying the pathogenesis of certain pulmonary lesions, especially emphysema and alveolar epithelial hyperplasia.

Introduction

Pulmonary lesions, resembling those seen in cattle dying of pulmonary adenomatosis, and death have been observed in dogs following intravenous injection of crystal violet. These observations were made while treating dogs with crystal violet for strongyloidosis at the Gentian violet dose level recommended for man (1). To further establish the toxicity of the drug and to determine if this observation might be used to study the pathogenesis of bovine pulmonary adenomatosis, additional animals were treated intravenously with crystal violet and methyl violet. The clinical and anatomical alterations produced by these dyes are described in this paper.

Materials and Methods

Nine dogs of mixed breeding (4-5 months of age) and 2 Holstein-Friesian calves (6 months of age) were inoculated intravenously with crystal violet[1] and/or methyl violet[2]. The dyes were prepared in saturated (1 per cent) unfiltered aqueous solutions. They were administered to the dogs at irregular intervals and in varying amounts (Table I). The calves were given the dyes at intervals of 1 to 2 days. The amount was gradually increased (25 ml. average) from 25 to 400 ml. Calf number 1, which lived for 28 days after receiving dye, was given 19 doses of crystal violet, making a total of 3,325 ml. Calf number 2 was given 2,950 ml. of methyl violet in 16 doses over a period of 30 days.

Since multiple amorphous pulmonary emboli were found in the lungs of animals administered unfiltered solutions, 3 additional dogs (10-12 in Table I) were administered a filtered solution of crystal violet (1 per cent) to determine if pulmonary emboli would occur as had been observed with the unfiltered solutions. Heparinized blood for paper electrophoresis of plasma proteins was obtained from these 3 dogs prior to the administration of crystal violet and again shortly before death.

Bovine serum and heparinized plasma were mixed with varying concentrations of crystal violet and methyl violet at 25 and 37 C to determine if a precipitate would form. Gential violet (1 per cent) was mixed with bovine serum at 25 C.

The type and amount of protein precipitated by crystal violet were determined by paper electrophoresis of the supernates using a Beckman model R electrophoresis cell and analytrol.[3] Veronal buffer (pH 8.6 and ionic strength 0.075), 0.1% bromphenol blue, and Schleicher and Schuell filter paper strips were used. The electrophoresis was carried out at a constant current of 2.5 milliamperes per 8 paper strips and 22 C for 16 hours.

Necropsies were performed shortly after death. Tissues were selected for microscopic examination, fixed in 10 per

*National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Ames, Iowa.

[1] Fisher Scientific Company, Fair Lawn, N.J. The optical density of the crystal violet used was 0.79 at 587 millimicrons and 8 milligrams per liter. Optical densities of crystal violet of other sources varied from this as much as plus 52 per cent and minus 30 per cent at the same wave length and concentration.
[2] Fisher Scientific Company, Fair Lawn, N. J.
[3] Beckman Instruments, Inc., Spinco Division, Palo Alto, California.

Table I. Intravenous administration of 1 per cent solutions of methyl violet and crystal violet to dogs.

Canine number	Dye	Dose (ml.)	Number of doses	Days at each dose level	Days from last dose until death
1.	MV*	10	5	36	< 1
	MV	20	1	1	
2.	MV	10	3	10	2
3.	MV	10	10	53	< 1
	MV	40	1	1	
4.	CV**	10	10	56	
	CV	15	1	8	< 1
	CV	20	1	1	
5.	CV	10	10	54	
	CV	20	2	9	7
6.	CV	10	6	20	
	CV	20	1	6	4
7.	MV	10	5	16	
	CV	20	1	1	1
8.	MV	10	5	16	
	CV	20	1	1	< 1
9.	MV	10	5	16	
	CV	20	2	20	14
10.	CV	10	2	3	2
11.	CV	10	2	3	2
12.	CV	10	2	3	2

*Methyl violet.
**Crystal violet.

TABLE II. Relative percentages of plasma proteins of 3 dogs before and after 2 intravenous treatments with 10 ml. of 1 per cent filtered aqueous crystal violet solution as determined by paper electrophoresis.

Dog number	Time obtained	Fibrinogen	Gamma globulin	Beta globulin	Alpha globulin	Almumin
10.	Pretreatment	20.1	12.2	7.9	6.1	53.6
	Posttreatment	16.4	19.0	16.4	10.3	37.9
11.	Preatreatment	17.4	13	6.5	6.5	56.5
	Posttreatment	22.4	13	13.6	9.5	40.8
12.	Pretreatment. . . .	22.4	8.2	8.2	10.1	50.1
	Posttreatment	16.8	10.1	23.4	10.2	39.6
Average.	Pretreatment	19.9	11.1	7.5	7.6	53.4
	Posttreatment	18.6	14.1	17.8	10.0	39.4

cent buffered formalin, embedded in paraffin, sectioned and stained with Harris' hematoxylin and eosin.

Results

All animals, except 2 dogs (5 and 9 in Table I) which were electrocuted in the terminal stages of toxicity, died from the effects of the dyes. Differences were not found between crystal violet and methyl violet poisoning or following the use of filtered or unfiltered solutions.

Canine Toxicity

Violet discoloration of the visible mucous membranes and lethargy follow-

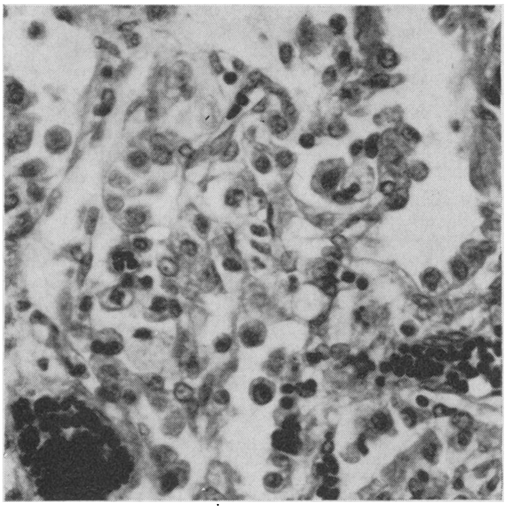

Fig. 1. Hyperplasia of alveolar epithelium and intraseptal macrophages, X500.

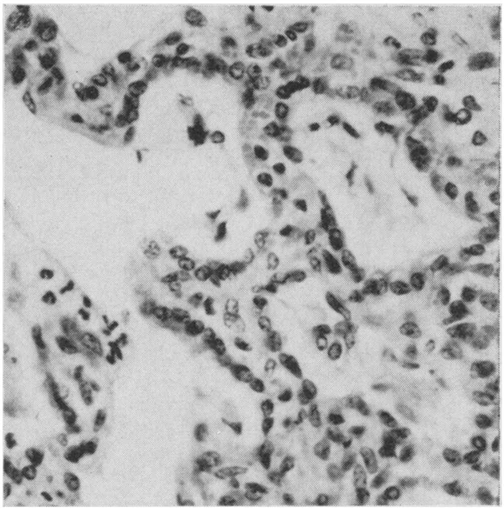

Fig. 2. Alveoli lined with hyperplastic epithelium, X320.

ed injections of the dyes. These signs had disappeared in 12 to 24 hours. There was a progressive increase in the respiratory rates throughout the course of the experiment until the terminal stages when abdominal breathing and, in some instances, expiratory grunting occurred. Respiratory rates were most accentuated immediately following injection of the dyes. Vomiting and diarrhea became prominent clinical signs prior to death. Feces were violet in color.

Tissues, especially the pancreas, diaphragm, and mucosa of the intestines, and the bile and intestinal contents of dogs that did not survive 1 day (Table I) following injection of the dyes, were various shades of violet. Most of the dye that remained in the tissues was extracted by the alcohol used in dehydrating specimens for histologic preparations.

The lungs were enlarged and contained many consolidated areas that were most numerous in the antero-ventral portions. Hemorrhage and edema were found throughout the pulmonary tissue. Alveolar septa were thickened as a result of edema, hemorrhage and hyperplasia of intraseptal macrophages (Fig. 1). Hyperplastic alveolar epithelium lined many alveoli and slight alveolar emphysema was present (Fig. 2). Many alveoli and bronchioles contained fibrin, macro-

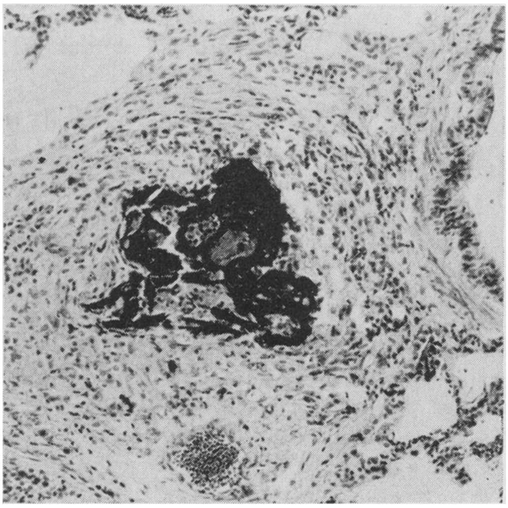

Fig. 3. Dye-protein coagulum obstruction in an artery, X125.

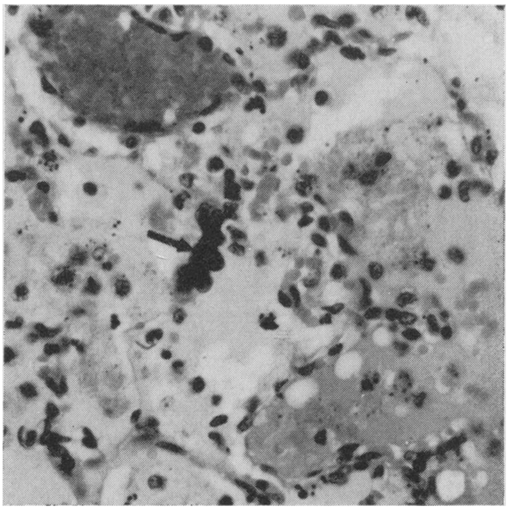

Fig. 4. Dye-protein coagulum in a capillary (arrow). The alveoli are filled with edematous fluid, fibrin, and erythrocytes, X320.

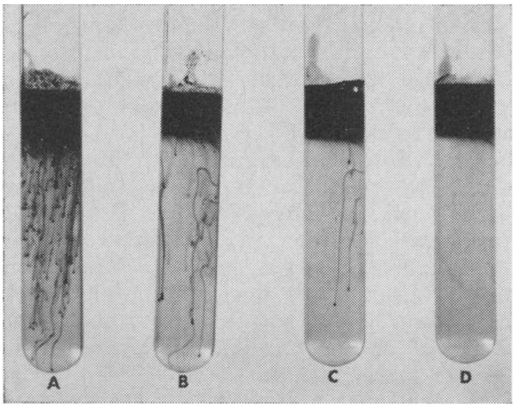

Fig. 5. Precipitate that was settling 1 minute after the addition of 0.5 ml. of (A) 1 per cent, (B) 0.5 per cent, (C) 0.25 per cent, and (D) 0.125 per cent aqueous crystal violet solutions to 10 ml. of heparinized bovine plasma.

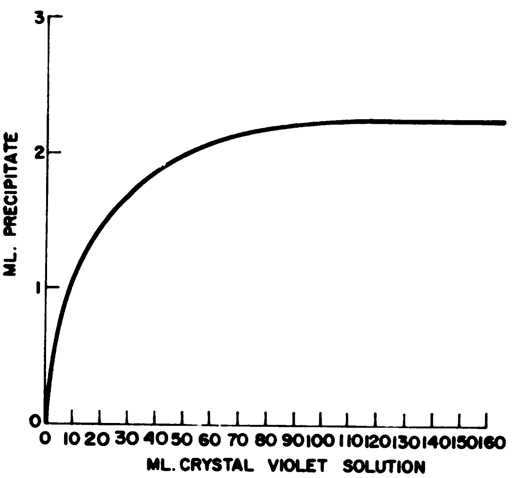

Fig. 6. Precipitate formed following addition of filtered crystal violet (1 per cent) in distilled water to 5 ml. of bovine serum. The mixture was incubated at 25° C for 1 hour, centrifuged at 2,000 rpm for 20 minutes, and the amount of precipitate determined.

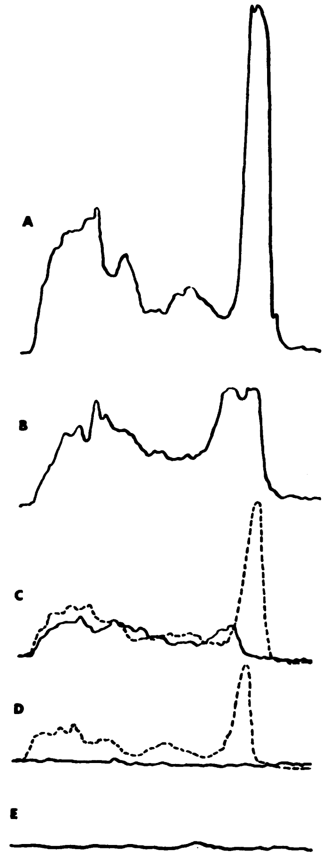

Fig. 7. Electrophoretic patterns of (A) normal bovine serum and supernatants from mixtures of bovine serum and 1 per cent aqueous crystal violet solution in ratios of (B) 5 to 1, (C) 1 to 1 (unbroken lines), and (D) 1 to 2 (unbroken line), and mixtures of bovine serum and distilled water in ratios of (C) 1 to 1 (broken line), and (D) 1 to 2 (broken line), and of (E) 1 per cent crystal violet in distilled water.

phages, and an occasional neutrophil. Some of the alveolar and bronchial exudate was organized. A dye-protein coagulum obstructed many arteries (Fig. 3) and caused thrombosis, infarction and chronic arteritis. Many capillaries were obstructed with the same coagulum (Fig. 4). A fibrino-necrotic pleuritis involved extensive areas of the visceral pleura. Fibrinous adhesions were present between the visceral and parietal pleura and the pericardium. The necrotic pleura and subpleural tissues, especially at the ventro-lateral edges of the lungs, contained a few mineralized foci. Some of the necrotic areas were partially organized. A general passive hyperemia was present.

The sinuses of many lymph nodes, especially those draining the lungs and intestines· contained blood. Macrophage hyperplasia and lymphocyte depletion were found in some nodes. The sinuses of the spleen were engorged with blood and the splenic parenchyma was depleted of lymphocytes.

The renal tubular epithelium was swollen and the lumen of the tubules contained lymphocytes, cellular debris, and albumin. Albuminous strands and globules were present in the glomerular spaces and the glomeruli were infiltrated with a few lymphocytes. Diffuse cloudy swelling together with peripherocentral fatty degeneration of the hepatic parenchyma were present. In some instances the hepatic triads were infiltrated with lymphocytes and macrophages.

Bovine Toxicity

Clinical signs, except for the absence of vomiting, and most anatomic alterations in cattle were similar to those observed in the dogs. The principal anatomic alteration that differed from lesions observed in the dogs consisted of a marked alveolar and interstitial pulmonary emphysema. Bronchial and mediastinal lymph nodes were distended with gas. The central nervous system contained multiple microscopic hemorrhages.

Pathogenesis

Saturated (1 per cent) aqueous solutions of crystal violet and methyl violet caused precipitation of both bovine serum and plasma **in vitro**. Gential violet caused formation of a precipitate when mixed with bovine serum. When 0.5 ml. of 1 per cent, 0.5 per cent, or 0.25 per cent aqueous crystal violet solutions was added to 10 ml. of bovine plasma, an amorphous precipitate formed but did not occur when 0.125 per cent crystal violet was added (Fig. 5). A direct relationship existed between the volume of dye solution added and the volume of precipitate formed (Fig. 6). Paper electrophoresis of the supernatant revealed that serum proteins in general were precipitated by the dye but were not precipitated when serum was diluted with distilled water (Fig. 7).

Paper electrophoresis of canine serum and plasma showed a relative decrease in the per cent of albumin after the administration of crystal violet. Fibrinogen was also decreased in 2 of the 3 dogs and in all 3 there was a relative increase in the amount of globulins (Table II).

Discussion

This study confirmed previous observations that crystal violet can cause fatal pulmonary alterations in dogs. These changes were not specific for crystal violet but also occurred with methyl violet. Gentian violet would probably produce a similar response since it is essentially a mixture of crystal violet and methyl violet (1) and did cause precipitation of bovine serum *in vitro*.

The dyes were administered at irregular intervals and in varying amounts depending on the individual tolerance of the animals. If given too frequently or in too large an amount, death would result before chronic pulmonary alterations were produced.

The lesions observed in this study were the result of multiple dye-protein emboli which lodged in the lungs and produced thrombi in the arteries and capillaries. Plasma protein alterations occurring in dogs during administration of crystal violet resulted from the formation of dye-protein emboli and exudate in the lungs.

Alterations produced in cattle were similar to those observed in dogs except for the presence of pulmonary emphysema. This difference in susceptibility to emphysema is a characteristic of the bovine lung and is thought to result from the greater pressures exerted on the lungs of cattle than dogs during dyspnea, together with differences in pulmonary structure.

The alveolar epithelial hyperplasia represents a nonspecific sequela to infarction, prolonged edema, fibrin deposition, and passive hyperemia. Spencer (2) has reported similar hyperplasia in man following pulmonary infarction from other causes. The degenerative alterations in hepatic and renal tissues were probably secondary to prolonged hypoxia.

The violet color of the bile indicated that the liver was the primary organ for elimination of the dyes. Staining of the intestinal mucosa was caused by the presence of dye eliminated through the biliary system.

Although these dyes do not produce lesions identical to those found in naturally occurring cases of bovine pulmonary adenomatosis, they could be used to advantage in determining the pathogenesis of some of the lesions. They would be especially applicable to a study of hyperplasia of the alveolar epithelium.

ACKNOWLEDMENTS

The authors thank L. Elliott, D. Wilson, R. Moore, and J. Heminover for technical assistance and R. Glazier and staff for assistance in photography.

REFERENCES

1. OSOL, A. and FARRAR, G. E. United States Dispensatory. J. B. Lippincott, Philadelphia, 24th ed., pp 710-711. 1947.
2. SPENCER, H. Pathology of the lung. The McMillan Co., New York, pp 458-463. 1962.